# FROM 2022 for consideration

**Prep required of participants before course:**

* **Complete introduction tutorials:**

1. Shell and command line coding: <https://learn.datacamp.com/courses/introduction-to-shell> - Chapter 1
2. R and R studio:[https://learn.datacamp.com/courses/free-introduction-to-r - Chapter 1](https://learn.datacamp.com/courses/free-introduction-to-r%20-%20Chapter%201)

# Monday

### Introduction to Course – 30 minutes

Learning objectives – Paul

Housekeeping – Paul

Instructor Introductions – Paul

### Introduction to Microbial Ecology Research in Life Sciences – 90 minutes

Research questions that people ask – Paul

Background of molecular stuff that generates the data (molecular workflow) – Paul

[Avoid talking about the microbial community metrics in this section]

End result is SEQUENCE DATA – describe fastq file – Paul

### BREAK – 15 minutes

### Navigating Server – 90 minutes

* Lecture: Intro by Peter.
  + WHY is this important?
  + WHAT is it?
* Live demo
  + Transferring data to remote server
  + Exploring file system
  + Show important variables that determine facts about the system's configuration
  + Manipulating files and directories
    - Wildcard and expansion
    - Text editors
    - Show fastq file
* Small-group tasks
  + Gathering (linking or copying) files
  + Changing permissions
  + Transferring files server to local
  + Sending someone file path

### Managing Data – 30 minutes

* Lecture: Intro by Peter.
  + WHY is this important?
  + WHAT is it?
  + Software discussion – Installation and management
  + Tracking discussion – Git/GitHub discussion
  + Backing up discussion
* Live demo: Peter.
  + FileNaming\_conventions
  + Simple project setup

NOON BREAK – 45 minutes [potentially with student research questions]

### 16S Workflow – Lee – 3.5 hours

OVERVIEW

Lecture: What’s the 16S gene, why do we use it?

Lecture: data QC

* show a bunch of fastqc/multiqc files (good and bad)

Hands-on: fastqc, cutadapt, denoising, merging, trimming, generate ASV table

Lecture: 16S databases

Lecture: 16S classification algorithms

Hands-on: classification, +/- species-level step

End of Day Summary - Including what to think about overnight – 30 minutes

END OF DAY GOAL: Have all of the ASV Classification table, and associated metadata files, etc. Teachers give basic presentation of the results as demonstration.

Tuesday

PREVIOUS DAY REVIEW: 15 minutes

General approach mirrors Mon afternoon

* Shotgun Workflow
* AMR and Taxonomy will be teaching examples for Shotgun, but discuss other classification targets.
* Classification approaches

MORNING – Noelle (lecture) and Enrique (hands-on)

01 Lecture: Comparison to 16S workflow

02a Lecture: Short lecture on server considerations for large datasets?

02b Lecture: QC

Hands-on: QC

Lecture: Host removal

Hands-on: Host removal

Lecture: Databases

Lunch: 45 minutes

AFTERNOON – Noelle (lecture) and Enrique (hands-on)

Lecture: microbiome classification for metagenomic data (classification, databases)

Hands-on: kraken

Lecture: resistome analysis for metagenomic data (classification, databases)

Hands-on: resistome

Lecture: other pipelines/approaches

End of Day Summary - Including what to think about overnight (teachers give basic examples of results as demonstration point) – 30 minutes

END OF DAY GOAL: Count matrices for ARGs and for KRAKEN,

Wednesday

PREVIOUS DAY REVIEW:

MORNING

Participants describe their research – 3MT – 90 minutes (volunteers)

* R introduction
* Ecological concepts and actual statistics
* Relative abundance, NMDS, Box Plot

Lecture/Hands-On combined: R programming and RStudio introduction, loading data, basic functions – Enrique – 45-60 minutes

Lecture/Hands-On combined: qubit concentrations, 16S qPCR counts, number of reads per sample, positive and negative controls, seq depth (rarefaction), batch effects, etc… – Noelle –

90-105 minutes

AFTERNOON

Lecture: concepts around microbiome data and statistical considerations (normalization, transformation, sparseness, compositionality, dimension reduction) – Enrique

Ecological concepts, statistical tests, and data viz:

Lecture: initial data exploration – Lee

Hands-on: relative abundance plots – Lee

Lecture: alpha diversity – Lee

Hands-on: alpha diversity – Lee

Lecture: beta diversity – Lee

Hands-on: beta diversity – Lee (including MicroViz)

Lecture: differential abundance – Enrique

Hands-on: differential abundance – Enrique (including DAtest)

End of Day Summary - Including what to think about overnight:

END OF DAY GOAL: Basic Alpha and Beta Diversity, both 16S and Shotgun ARG/KRAKEN

Homework Assignment: Survey/Email with Questions for Thursday Morning

Thursday

9-10am Answer submitted questions/group discussion

10-10:15am Break

10:15-12pm Journal club on Animal Microbiome rumen paper

12-1pm Lunch

1pm-4pm Small group research questions and data analysis

Friday

8:30-11:30 Work time in small groups

11:30-12:30 Lunch

12:30-2:30 4 group presentations

2:30-2:45 BREAK

2:45-4:15 3 group presentations

4:15-5pm Ask-the-instructor and wrap-up

[Placeholder]: How to design microbiome study?

[Placeholder]: research best practices

[Placeholder]: ecology concepts